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Generation of S-Layer Supported Functionalized Lipid Bilayers

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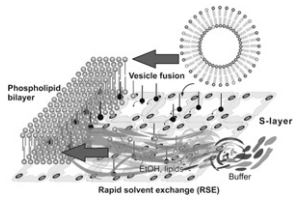
Protein-stabilized functional lipid membranes on solid supports are a preferred target for pharmaceutical and technological applications. The vast interest of such systems is attributed to the fact that nearly one third of all proteins are membrane proteins.

In our study we present the characterization of bacterial surface layer (S-layer) supported lipid bilayers as well as the insertion of membrane proteins or peptides. Common methods to build up a lipid double layer structure are by the rapid solvent exchange (RSE) mechanism or by vesicle fusion. Both techniques lead to a dense lipid bilayer. One approach in this study is the direct insertion of channel forming peptides which has been determined by several surface sensitive and biophysical methods. The second approach is the live extraction and transfer of membrane fragments from living cells by so called smart droplet micro (SDM) tools within an optical tweezer manipulation set up. The main results advocate that the physical and chemical properties of S-layers and slightly chemically modified S-layers trigger lipid membrane generation as well as the insertion of membrane proteins. Overall, S-layers provide a useful scaffold for biomimetic membranes due to its functional properties and biological nature.



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Surface Electrostatics Associated with Lipid Bilayer Curvature

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Spontaneous lipid bilayer bending and related curvature are being recognized as an essential mechanism associated with many cellular functions. Experimental observations indicate that tubular vesicles of just 50 to 100 nm in diameter are commonly formed within the Golgi body and also between the endoplasmic reticulum and the Golgi. While changes in various conditions may promote the tubulation of the Golgi membrane, detailed understanding of basic biophysical processes beyond the bilayer bending are missing in the literature and so are convenient models of highly curved lipid tubules. Here we report a systematic study of a surface potential for both small unilamellar monodisperse lipid vesicles (SUMV) with diameters ranging from 30 to 100 nm and also lipid tubules that are stabilized by confining these structures within rigid homogeneous nanopores of similar diameters. For example, for SUMVs composed of negatively charged lipids the magnitude of the surface potential increased with bilayer bending from ca. −106 mV for 100 nm SUMV to −166 mV for 30 nm SUMV. These measurements were carried out by spin probe EPR method using recently synthesized lipids having pH-reporting nitroxides covalently tethered to the lipid polar head. Parallel differential scanning calorimetry experiments indicated the presence of at least two components within the lipid phase. These phase components were characterized by measurably different phase transition temperatures and correlation times of the lipid thermal relaxation. Overall, the data indicate that the bilayer bending affects the local electrostatic potential and lipid fluctuation properties in a rather large degree and is likely associated with a mechanism for cellular machinery function. Supported by U.S. DOE Contract DE-FG02-02ER15354.

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Electrokinetic Determination of the Surface Potential of Tethered Lipid Bilayers

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The surface potential of a membrane is involved in a number of important physiological processes such as fusion, recognition, and solute binding. Artificial models such as tethered lipid bilayers enable us to capture several key properties of complex biological lipid membranes such as their lateral dynamics, molecular structure, and interactions with peripheral proteins. Membrane electrostatics represents one further aspect for these mimics to address. Here we demonstrate how the surface potential of tethered phospholipid bilayers may be electrokinetically determined with accuracy of a couple mV, by measuring the membrane streaming potential in a well-defined laminar flow. Surface potentials have been determined for a variety of lipid compositions, and compared to those obtained from the conductance of black lipid membranes and the electrophoretic mobility of unilamellar vesicles.

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The Combined Effect of Electrostatic Field and Osmotic Pressure on the Stability of Bilayer Lipid Membranes

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The issue of cell membrane stability is central in membranology. The extreme complexity of cell membranes urges to study this problem using a model, a bilayer lipid membrane (BLM). The membranes is often impacted both by electric forces and osmotic pressure. Therefore, it is of interest to investigate the joint activity of electric field and osmotic pressure on the stability of BLM. In the given work the joint activity of the osmotic pressure and transmembrane potential difference on the stability of BLM was experimentally investigated. As the capacity parameter a mean lifetime of BLM was taken at the given magnitudes of electric field and the osmotic pressure.

At first the change of the mean lifetime of BLM with increasing of potential difference in absence of osmotic pressure was investigated. It was shown that the activity of electrostatic field brings decreasing of BLM mean lifetime. After the influence of potential difference on BLM mean lifetime was experimentally investigated at addition of osmotic pressure. It was shown that a dependence curve of BLM mean lifetime with potential for the second case is lower than in the first case. The loss of stability of BLM at addition of osmotic pressure is conditioned by two factors: either changing linear tension of BLM pore, changing the numbers of defects on BLM. The analysis theoretical formula and its confrontations with obtained data shows that the decreasing of mean lifetime of BLM at addition of osmotic pressure can be connected with the changing of linear tension. It is indicated that the difference in osmotic pressure of BLM does not bring to increasing of an area.

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Thermodynamics of Chlorpromazine Association with Lipid Bilayers

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Passive transport across cell membranes is the major route for the permeation of xenobiotics through tight epithelia, such as the vascular endothelium that constitutes the Blood Brain Barrier. The rate of passive permeation through lipid bilayers for a given drug is therefore a critical step in the prediction of its *in vivo* efficacy. This parameter is usually evaluated from the drug hydrophobicity with little consideration for the rate of interaction and/or translocation through the lipid bilayer. Our research group is developing a global model to quantitatively describe the rate of passive permeation through tight endothelia and one of the outcomes is that in most cases the rate of the interaction (rather then the equilibrium partition) is the most relevant parameter.

In this work we present a detailed study on the kinetics and thermodynamics for the interaction of chlorpromazine (CPZ) with neutral and negatively charged lipid bilayers. CPZ is an antipsychotic drug used in the treatment of schizophrenia and is an ideal candidate for studies of passive permeation of the BBB by active drugs.

The partition and translocation of CPZ in lipid membranes was studied by Isothermal Titration Calorimetry (ITC) and the lipid bilayers were composed of pure POPC, POPC:POPS (9:1) and POPC:Chol:POPS (6:3:1). The charge imposed by CPZ in the membrane was taken into account allowing the calculation of the intrinsic partition coefficients (K_P) and the enthalpy change (ΔH) associated with this process. The relative rate of the partition and translocation processes was obtained following the uptake and release protocol [1]. A quantitative evaluation of the rate of translocation is also given for some systems using an innovative methodology.

[1] Tsamaloukas, A. D., Keller, S. and Heerklotz, H. (2007) *Nat. Prot.* 2, 695-704

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Halogenated Anesthetics Impairs Biophysical Properties of a Membrane Model of Pulmonary Surfactant

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Pulmonary surfactant (PS) is a complex mixture of lipids and proteins responsible to protect the lung tissue from mechanical stress during ventilation, by decreasing alveolar surface tension. Indeed, an appropriate PS function is critical in order to avoid alveolar collapse. There are many causes that produce PS dysfunction, lung damage and alveolar collapse. Our aim was to explore the effect of Halogenated Anesthetics on functional PS. Sevoflurane is widely used in different protocols of anesthesia. However, there is little evidence of the mechanisms that could explain the damage in alveolar space observed in animal models (1). In the present study we evaluate the effect of Sevoflurane in a mixture of synthetic lipids (50:50, DPPC:PC and 20% mol Cholesterol) as a model